REMARKS/ARGUMENTS

The Pending Claims

Claims 1, 5, 7-11, 14, and 34-41 currently are pending and subject to examination.

Amendments to the Claims

As a preliminary matter, Applicants note that the application was filed with claims 1-33. In the response to the first restriction requirement filed on January 20, 2007, Applicants indicated that claims 1-3, 5-11, and 14 are pending and that claims 4, 12, 13, and 15-30 are canceled. Applicants did not discuss the disposition of claims 31-33, which were intended to be canceled. This oversight was carried throughout prosecution, and in the "Reply to Office Action" filed on January 25, 2010, Applicants entered two new claims numbered as claims 31 and 32, when, in fact, these new claims should have been numbered as claims 34 and 35. The misnumbered claims 31 and 32 presented on January 25, 2010, further amended as described below, have been renumbered and are presented herein as new claims 34 and 35.

The claims have been amended to point out more particularly and claim more distinctly the invention. In particular, claim 1 has been amended to specify that the refolding buffer comprises PEG and/or lauryl maltoside, as supported by the specification at, e.g., paragraphs 0034 and 0167. New claims 34 and 35 differ from misnumbered claims 31 and 32 presented on January 25, 2010, by specifying that the refolding buffer comprises PEG and/or lauryl maltoside.

New claims 36, 38, and 40 specify that the refolding buffer of claims 1, 34, and 35, respectively, comprise both PEG and lauryl maltoside. New claims 37, 39, and 41, specify that the refolding buffer of claims 36, 38, and 40, respectively, comprise about 0.02-10 mM GSH, 0.005-10 mM GSSG, 0.005-10 mM lauryl maltoside, 50-250 mM NaCl, 2-10 mM KCl, 0.01-0.05% PEG 3350, and 150-550 mM L-arginine. New claims 36-41 are supported by the specification at, e.g., paragraphs 0045 and 0167.

Claim 5 has been amended to correct grammar.

No new matter has been added by way of these amendments.

Information Disclosure Statements

Since Applicants are filing a Request for Continued Examination (RCE) herewith, Applicants respectfully request consideration of (a) the previously filed Information Disclosure Statement dated September 20, 2010, and the references identified and submitted therewith, in accordance with M.P.E.P. § 609 (see, e.g., M.P.E.P. § 609.02(B)(3)), and (b) the Information Disclosure Statement and accompanying references submitted herewith.

The Office Action

Claims 1, 5, 7-11, 14, 31, and 32 have been rejected over U.S. Patent 5,858,751 (Paulson et al.) in view of Hellman et al., (*Prot. Expr. Purif., 6*: 56-62 (1995)) and Clark (*Cur. Opin. Biotech., 12*: 202-207 (2001)), alone or further in view of Ramakrishnan et al. (*J. Biol. Chem., 276*: 37665-37671 (2001)) or Nilsson et al. (*Prot. Expr. Purif., 11*: 1-16 (1997)).

Reconsideration of this rejection is respectfully requested in view of the claim amendments and remarks herein.

Discussion of the Obviousness Rejection

The Office alleges that the subject matter defined by claims 1, 5, 7-11, 14, 31, and 32 (now claims 1, 5, 7-11, 14, 34, and 35) was obvious over Paulson in view of Hellman et al. and Clark, alone or further in view of Ramakrishnan et al. or Nilsson et al. According to the Office, it would have been obvious to one of ordinary skill in the art to have used the methods and reagents disclosed in Hellman et al., Clark, Ramakrishnan et al., and/or Nilsson et al. to refold the eukaryotic α(2,3)sialyltransferase (ST3Gal3) disclosed in Paulson et al.

For subject matter defined by a claim to be considered obvious, the Office must demonstrate that the differences between the claimed subject matter and the prior art "are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains." 35 U.S.C. § 103(a); see also *Graham v. John Deere Co.*, 383 U.S. 1, 148 U.S.P.Q. 459 (1966). The ultimate determination of whether an invention is or is not obvious is based on certain factual inquiries including: (1) the scope and content of the prior art, (2) the level of ordinary skill in the prior art, (3) the differences between the claimed invention and the prior

art, and (4) objective evidence of nonobviousness. *Graham*, 383 U.S. at 17-18, 148 U.S.P.Q. at 467.

Consideration of the aforementioned *Graham* factors here indicates that the present invention, as defined by the amended claims, is unobvious in view of the cited references.

1. Scope and Content of the Prior Art

Paulson et al. discloses methods of producing biologically active mammalian α(2,3)sialyltransferase (ST3Gal3) in recombinant host cells. In particular, Paulson et al. discloses a method of producing a biologically active fusion protein between the catalytic domain of rat α(2,3)sialyltransferase and the insulin signal sequence in mammalian cells (COS-1 cells) (column 30, line 35 – column 31, line 30). Paulson et al. also discloses a method of producing a biologically active, truncated mammalian α(2,3)sialyltransferase lacking the signal sequence and transmembrane domain in insect cells (Sf-9 cells) (column 31, line 48 – column 33, line 42).

Hellman et al. discloses a method to produce a biologically active fusion protein between a bacterial cyclomaltodextrin glucanotransferase and maltose-binding protein following expression in a prokaryotic host cell (*E. coli*) by refolding the fusion protein obtained from inclusion bodies using a MOPS-based buffer system comprising a gradient of urea or guanidinium chloride (GdmCl). Hellman et al. recognizes the difficulties of obtaining biologically active protein from bacterial inclusion bodies, stating that "[e]ach protein seems to require a specific denaturation-refolding pathway to give a maximal yield of functional molecules" (page 56, right column).

Clark is a review article that describes general strategies for obtaining biologically active protein from bacterial inclusion bodies. In particular, Clark discloses the use of refolding buffers having extremes of pH or refolding buffers comprising (i) denaturants such as GdmCl or urea, (ii) detergents such as sodium dodecyl sulfate (SDS) or n-cetyl trimethylammonium bromide (CTAB), (iii) reducing agents such as dithiothreitol and 2-mercaptoethanol, (iv) redox pairs such as GSH/GSSG and cysteine/cystine, and (v) low molecular weight additives such as L-arginine. Clark recognizes the complexities of obtaining biologically active protein from prokaryotic expression systems, stating that despite

the guidelines provided in the review article, "optimum conditions have to be determined on a cases by case basis" (page 206, left column).

Ramakrishnan et al. discloses that a cysteine to threonine mutation at amino acid residue 342 of β-1,4-Galactosyltransferase 1 (Gal-T1) enhances *in vitro* folding of Gal-T1 from inclusion bodies and increases its stability without perturbing the crystal structure of the enzyme. The refolding buffer disclosed in Ramakrishnan et al. contains Tris-HCl, GdnHCl, EDTA, cysteamine, and cystamine (page 37666, left column).

Nilsson et al. discloses the fusion of one or more affinity tags selected from MBD, starch binding domain, thioredoxin domain, GST domain, and poly-histidine domain to a protein of interest in order to facilitate protein purification.

2. Level of Ordinary Skill in the Art

For purposes of the analysis here, and for the sake of argument, the level of ordinary skill can be considered to be relatively high, such that a person of ordinary skill in the art would have an advanced degree and/or several years of experience in the relevant field.

3. Differences Between Claimed Invention and Prior Art

The present invention is based, at least in part, upon Applicants' discovery of a novel method of refolding an insoluble eukaryotic ST3Gal3-MBD fusion protein to obtain a biologically active ST3Gal3 enzyme. This novel method is defined by amended claims 1, 34, and 35, which recite, *inter alia*, the use of a refolding buffer comprising a redox couple and PEG and/or lauryl maltoside.

Paulson et al. does not disclose a ST3-Gal3-MBD fusion protein or the use of any refolding buffer, much less a refolding buffer comprising a redox couple and PEG and/or lauryl maltoside. Although Paulson et al. discloses, generally, that "[p]rokaryotes are also used for expression" (column 11, line 32), Paulson et al. does not disclose or suggest any method to produce a biologically active ST3Gal3 enzyme from an insoluble, recombinant ST3Gal3 protein.

Hellman et al. discloses refolding conditions for an insoluble, recombinant prokaryotic enzyme, namely cyclomaltodextrin glucanotransferase. The refolding buffer disclosed in Hellman et al. does not comprise a redox couple, PEG, or lauryl maltoside.

Clark discloses a laundry list of reagents suitable for use during the refolding of proteins obtained from bacterial inclusion bodies. Clark does not disclose or suggest a specific refolding protocol for any glycosyltransferase, much less ST3Gal3, specifically. Moreover, Clark does not disclose the use of any refolding buffer comprising PEG or lauryl maltoside.

Ramakrishnan et al. discloses refolding conditions for Gal-T1, but Ramakrishnan et al. does not disclose or suggest a refolding buffer comprising PEG or lauryl maltoside.

Nilsson et al. does not disclose a refolding buffer for any glycosyltransferase, much less a buffer comprising a redox couple, PEG, and/or lauryl maltoside to produce biologically active ST3Gal3.

Thus, none of the cited references discloses a method of refolding an insoluble eukaryotic ST3Gal3-MBD fusion protein to obtain a biologically active ST3Gal3 enzyme using a refolding buffer comprising a redox couple and PEG and/or lauryl maltoside.

4. Objective Evidence of Unobviousness

For purposes of the present argument, Applicants have no need to refer to any objective evidence of unobviousness of the present invention as defined by the amended claim.

5. Consideration of Graham Factors Together

The combined disclosures of the cited references do not reflect all of the limitations of the pending claims, and one of ordinary skill in the art would not necessarily arrive at the present invention by following the combined teachings of the cited references. Applicants acknowledge that, in general, PEG and lauryl maltoside have been described in the art as suitable components for use during the refolding of recombinant proteins. However, Applicants are unaware of any reference which discloses the use of PEG and/or lauryl maltoside to facilitate refolding of any glycosyltransferase, much less ST3Gal3 specifically. Moreover, none of the references cited by the Office provide any credible reason for one of ordinary skill to modify the refolding conditions disclosed therein to include PEG and/or lauryl maltoside, much less provide any expectation of success in obtaining a biologically active ST3Gal3 enzyme by the inclusion of PEG and/or lauryl maltoside.

A claimed invention composed of several elements is not proved to be obvious merely by demonstrating that each element was, independently, known in the prior art. *KSR Int'l v. Teleflex, Inc.*, 550 U.S. 398, 418, 82 U.S.P.Q.2d 1385, 1396 (2007). The Supreme Court has held that, in a proper analysis, one must still "determine whether there was an apparent reason to combine the known elements in the way a patent claims." *Id.*

Thus, in *KSR*, the Supreme Court acknowledged the importance of identifying "a reason that would have prompted a person of ordinary skill in the relevant field to combine the elements in the way the claimed new invention does." 550 U.S. at 418, 82 U.S.P.Q.2d at 1396 (2007). Here, the Office has failed to identify any credible reason why one of ordinary skill in the art would have "picked and chosen" the particular elements disclosed in the cited references identified by the Office (as opposed to the many other elements disclosed in the cited references) and then combined those elements in the manner necessary to arrive at the invention recited in the pending claims. Contrary to the assertions of the Office, Applicants submit that one of ordinary skill in the art – without hindsight knowledge of the present invention – would not have had a credible reason to use the methods and reagents disclosed in Hellman et al., Clark, Ramakrishnan et al., and/or Nilsson et al. to refold the eukaryotic α(2,3)sialyltransferase (ST3Gal3) disclosed in Paulson et al.

One of ordinary skill in the art understands that protein refolding is not a predictable science and that the development of a suitable method for refolding a specific protein such as ST3Gal3 requires a careful evaluation of numerous variables, including the precise components of the refolding buffer. In fact, this view is shared by the authors of two of the references utilized by the Office in support of the alleged obviousness of the claimed method. As discussed above, Hellman et al. states that "[e]ach protein seems to require a specific denaturation-refolding pathway to give a maximal yield of functional molecules" (page 56, right column), whereas Clark states that "optimum conditions have to be determined on a cases by case basis" (page 206, left column). Thus, even amongst those highly skilled in the field of protein refolding, there is a general consensus that the optimal conditions for refolding an insoluble recombinant protein such as ST3Gal3 cannot be identified by merely "picking and choosing" elements described in the prior art for the refolding of a distinct and unrelated protein.

Considering all of the *Graham* factors together, it is clear that the present invention would not have been obvious to one of ordinary skill in the art at the relevant time in view of the combination of cited references. Accordingly, the obviousness rejections based on Paulson et al. in view of Hellman et al. and Clark, alone or further in view of Ramakrishnan et al. or Nilsson et al. should be withdrawn.

New claims 36, 38, and 40 have been added to specify that the refolding buffer of claims 1, 34, and 35, respectively, comprises both PEG and lauryl maltoside. In addition, new claims 37, 39, and 41, specify that the refolding buffer of claims 36, 38, and 40, respectively, comprises about 0.02-10 mM GSH, 0.005-10 mM GSSG, 0.005-10 mM lauryl maltoside, 50-250 mM NaCl, 2-10 mM KCl, 0.01-0.05% PEG 3350, and 150-550 mM L-arginine. As discussed above, none of the cited references discloses or suggests the use of a refolding buffer comprising PEG and/or lauryl maltoside to obtain biologically active ST3Gal3 enzyme, much less a refolding buffer comprising the specific reagents at the specific concentrations recited in claims 37, 39, and 41. Accordingly, favorable consideration of new claims 36-41 is respectfully requested.

Conclusion

Applicants respectfully submit that the patent application is in condition for allowance. If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned attorney.

Respectfully submitted,

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